Behavioral Ecology Vol. 10 No. 4: 436–443

A test of alternative hypotheses for kin recognition in cannibalistic tiger salamanders

David W. Pfennig,[a] James P. Collins,[b] and Robert E. Ziemba[b]
[a]Department of Biology, University of North Carolina, Chapel Hill, NC 27599, USA, and
[b]Department of Biology, Arizona State University, Tempe, AZ 85287, USA

The function of kin recognition is controversial. We investigated the adaptive significance of kin discrimination in cannibalistic tiger salamander larvae, *Ambystoma tigrinum*. Previous laboratory experiments show that cannibals preferentially consume less related individuals. We hypothesized that this example of kin recognition (1) is a laboratory artifact, (2) is a by-product of sibship-specific variation in escape responses, because cannibals from families with rapid responses may be more likely to cannibalize slowly escaping non-kin, (3) is an epiphenomenon of species recognition, (4) functions in disease avoidance, because kin may be more infectious than non-kin, or (5) is favored by kin selection. We evaluated these five hypotheses by using laboratory and field experiments to test specific predictions made by each hypothesis. We rejected hypotheses 1–4 above because (1) kin recognition was expressed in the wild, (2) escape responses did not reliably predict whether a cannibal would ingest kin or non-kin, (3) kin recognition was not most pronounced in populations where tiger salamanders co-occur with other species of salamanders, and (4) non-kin prey were more likely than kin to transmit pathogens to cannibals. However, we established that the necessary condition for kin selection, Hamilton's rule, was met. Thus, our results implicate kin selection as the overriding reason that cannibalistic tiger salamanders discriminate kin. *Key words: Ambystoma tigrinum,* cannibalism, disease transmission, Hamilton's rule, kin discrimination, kin selection, salamanders. *[Behav Ecol 10:436–443 (1999)]*

Kin recognition, the differential treatment of conspecifics varying in genetic relatedness, has been documented in multiple animal and plant taxa (reviewed in Fletcher and Michener, 1987; Hepper, 1991; Pfennig and Sherman, 1995; Waldman, 1991). Although the catalyst to study kin recognition is ultimately interest in its evolutionary cause (Waldman, 1991), little is known about the fitness consequences of kin discrimination (reviewed in Blaustein et al., 1991; Gamboa et al., 1991; Sherman et al., 1997). This gap in our knowledge has provoked a spirited debate regarding the evolutionary role of kin recognition (Blaustein et al., 1991; Grafen, 1990; Sherman et al., 1997; Stuart, 1991) and has even led some to speculate that most organisms do not really recognize kin at all (Grafen, 1990).

Both adaptive and nonadaptive hypotheses have been proposed to explain kin recognition (Barnard, 1991; Blaustein et al., 1987; Carlin, 1989; Grafen, 1990; Pfennig, 1990; Pfennig et al., 1993; Waldman, 1991). In particular, kin recognition has been purported to be (1) an epiphenomenon of some other recognition system, (2) maintained by natural selection because it enhances the direct component of a discriminator's inclusive fitness (i.e., the genes contributed to the next generation by an individual via personal reproduction), or (3) maintained by natural selection because it enhances the indirect component of a discriminator's inclusive fitness (i.e., the genes contributed to the next generation by an individual indirectly by helping nondescendant kin; Brown, 1987). These three hypotheses differ from one another in the fitness benefits derived by discriminating individuals. The epiphenomenal hypothesis posits that discriminators do not benefit by their actions, whereas the two selective hypotheses differ in the type of benefit that discriminators accrue.

Address correspondence to D. W. Pfennig, Department of Biology, Coker Hall, CB#3280, University of North Carolina, Chapel Hill, NC 27599-3280, USA. E-mail: dpfennig@email.unc.edu.

Received 22 March 1998; revised 11 August 1998; accepted 6 January 1999.

Cannibalistic species are ideal for testing alternative hypotheses for kin recognition because kin discrimination may be particularly well developed in these species (Pfennig, 1997, 1999; Pfennig and Collins, 1993; Pfennig et al., 1993, 1994; Sadler and Elgar, 1994; Wade, 1980; Walls and Roudenbush, 1991). Indeed, parents refrain from preying on their own offspring but readily cannibalize less related young in numerous cannibalistic species, and in some species, even collateral, or nondescendant, kin are avoided (reviewed in Pfennig, 1997; but see Walls and Blaustein, 1995, and references therein).

There are at least five hypotheses to explain why cannibals avoid preying on kin (Table 1). The first three hypotheses are epiphenomenal hypotheses, whereas the last two are selective hypotheses that differ in the type of benefit (direct or indirect) that a discriminating cannibal receives.

First, kin recognition might be an artifact of laboratory conditions (Gamboa et al., 1991). In laboratory tests (e.g., Pfennig et al., 1994), cannibals are given a choice of eating equal numbers of kin and non-kin. Kin recognition may be absent in more complex natural settings, where environmental heterogeneity and more diverse prey choices might preclude a cannibal's distinguishing kin from non-kin. Second, kin recognition might be a by-product of sibship-specific variation in escape responses. Cannibals from different sibships might vary in both the speed they attack prey and the speed of reaction to attacks from other cannibals. Thus, cannibals from families with rapid responses would be more likely to cannibalize slowly escaping non-kin than rapidly escaping kin, giving the spurious impression that cannibals prefer to eat non-kin. Third, kin recognition might be an epiphenomenon of species recognition (Grafen, 1990). In laboratory choice tests (Pfennig et al., 1994), cannibals are often raised with siblings only. Thus, they might learn their species recognition cues or "template" from siblings. If so, their avoidance of siblings might represent attempts to avoid consuming conspecifics. Indeed, when given a choice of preying on conspecific or heterospecific salamander larvae, tiger salamanders prefer to eat the latter (Pfennig et al., 1998).

Fourth, kin recognition might function in disease avoidance

Table 1
Hypotheses for why cannibals avoid eating kin

Hypotheses	Prediction(s)	Outcome of this study
1. Lab artifact	Kin discrimination should be absent in the wild	Rejected
2. By-product of sibship-specific variation in escape responses	(a) Individuals from different sibships should vary in escape response	Accepted
	(b) These differences should reliably predict which individual is cannibalized	Rejected
3. Species recognition	Kin discrimination should be most pronounced in areas where tiger salamanders are sympatric with other species of salamanders	Rejected
4. Disease avoidance	(a) Sibships should differ in susceptibility to disease	Accepted
	(b) Kin recognition should be most pronounced in diseased populations	Rejected
	(c) Kin should be more infectious than non-kin	Rejected
	(d) Cannibals should prefer to eat nondiseased prey	Rejected
5. Kin selection	Hamilton's rule should be satisfied; i.e., $rb - c > 0$	Accepted

(Pfennig et al., 1993). There are numerous accounts of parasites being transmitted via cannibalism (reviewed in Pfennig et al., 1998; Polis, 1981). Parasites are often strongly host specific, apparently because of coevolution between parasites and hosts (reviewed in Freeland, 1983; Møller et al., 1993). Thus, genetically similar organisms may be especially likely to exchange parasites. For example, cannibalistic tiger salamander larvae are more likely to acquire pathogens from conspecifics than from heterospecifics (Pfennig et al., 1998). Similarly, close relatives may be more likely than nonrelatives to exchange parasites, owing to greater genetic similarity among close relatives and selection for host specificity and resistance to host immune defenses among pathogens. Indeed, there is evidence from bumblebees and humans that certain parasites are more highly transmissible among kin than among non-kin (Black, 1994; Shykoff and Schmid-Hempel, 1991). Thus, ingesting close relatives may be costly to cannibals because kin may be more infectious than non-kin.

Fifth, kin recognition might be maintained by kin selection (Hamilton, 1964). In particular, a cannibal that recognizes and avoids preying on kin may act in its own genetic self-interest by propagating genes shared with kin, including genes for kin recognition. In more precise terms, kin recognition will be selectively favored whenever Hamilton's rule is satisfied (Hamilton, 1964); i.e., whenever $rb - c > 0$, where r is the coefficient of relatedness between discriminator and its potential prey, c is the cost of the act in terms of future offspring production that the discriminator loses by not eating the prey, and b is the benefit of the act in terms of the extra offspring that noncannibalized prey gain. Thus, kin recognition may be favored because of the indirect fitness benefits that a discriminatory cannibal accrues.

We evaluated the above five alternative hypotheses for kin recognition in cannibalistic tiger salamanders (*Ambystoma tigrinum*), a species that often occurs in nature as a typical morph that feeds mostly on invertebrates and occasionally on other salamanders and as a physically distinctive cannibal morph that preys mostly on conspecifics (Collins et al., 1993; Powers, 1907). Cannibal morphs are produced when larvae are crowded with other salamanders (Collins and Cheek, 1983; Hoffman and Pfennig, 1999). In laboratory choice tests, cannibalistic larvae use sibship-specific olfactory signals to feed voraciously on nonrelatives but avoid eating close kin (Pfennig et al., 1994). They also are significantly more likely to express the cannibal phenotype in mixed sibship groups than in pure sibship groups (Pfennig and Collins, 1993). To determine which of the above five hypotheses explains these well-developed kin recognition abilities, we tested specific predictions made by each hypothesis (Table 1).

METHODS AND RESULTS

Experimental animals

We studied *A. tigrinum* larvae from the White Mountains of Arizona and West Lafayette, Indiana, USA (for locations and descriptions of White Mountains ponds, see Pfennig et al., 1994). For the White Mountains populations, we used larvae from 10 sibships whose parents or grandparents were captured from 6 ponds. Each pair of adults was kept in a 120-l aquarium until the female laid eggs. For the West Lafayette population, we used larvae from 10 sibships whose parents were from a single pond. Adults were captured at a drift fence as they approached the pond to breed. We assigned males and females to pairs and placed them inside small, mesh cages that were partially submerged in the breeding pond. After the female oviposited, the eggs were removed. Once the eggs from both populations had hatched, we randomly chose 10–20 groups of 15 larvae from each sibship. Groups were placed into separate aquaria (22 l) with 16 l of dechlorinated tap water. All larvae were reared under identical conditions: 22°–25°C water temperature and 14 h:10 h photoperiod. During rearing, water was changed weekly and animals were fed ad libitum live brine shrimp (*Artemia* sp.) daily. At 7 weeks after hatching we scored larvae as being typical or cannibal morphotypes (the latter are characterized as having an enlarged vomerine ridge and elongate teeth; Pedersen, 1991).

Experiment 1: Is kin recognition an artifact of the laboratory?

Methods

To determine if tiger salamanders recognize kin in a natural setting, we tested kin discrimination abilities of cannibals from three Arizona sibships when they were 7 weeks old (equivalent to the age of test cannibals in previous laboratory studies; Pfennig et al., 1994). The experiment was conducted in Dude Lake, a natural pond on the Mogollon Plateau of Arizona. This pond contains many cannibals, and it is free of salamander disease epidemics (Loeb et al., 1994; Pfennig et al., 1991a). To start the experiment, we positioned 20 cylindrical mesh cages (0.75 m wide × 1.3 m deep) in 0.3 m-deep water. The cages were made of mesh small enough (2 mm) to retain salamander larvae, but large enough to admit naturally occurring prey (e.g., plankton and aquatic insects).

In each of 18 enclosures, we placed 25 larvae: 1 cannibal and 6 typical morph larvae from each of 4 different sibships (24 typicals total). Six of these typical morph larvae were the cannibal's siblings; the remaining 18 were nonsiblings. Typical morph larvae within each enclosure were matched for size;

they were about half the snout-vent length (SVL) of the test cannibal. Typical morph larvae had been reared apart from their cannibal siblings since they were ≤ 2 weeks old; different sibships were completely unfamiliar with each other. In each of the two remaining enclosures, we placed six typical-morph larvae from each of the four sibships. These served as controls to determine if larvae from different sibships differed in mortality rate.

To keep track of kinship identities, we used a 26-gauge hypodermic needle to inject into the dorsal tail membrane of each larva a mixture of fluorescent pigment and oil (one part mineral oil to one part petroleum jelly). Once injected, the mark formed a thin strip (approximately 1 mm wide × 20 mm long) of red, yellow, pink, or orange fluorescent pigment. Within each enclosure, animals from different sibships were injected with different colors. These marks were visible under ordinary light until after metamorphosis and did not affect larval mortality. To control for any effects of different colors on a cannibal's prey preferences, the test cannibal's siblings were represented by each color type in different enclosures.

The experiment began on 10 August 1994. An observer (unaware of the sibship identities of the stimulus animals) checked each enclosure approximately weekly and noted the tail marks of surviving stimulus animals. We inferred that cannibalism had occurred if a larva was missing. Censuses were conducted on 20 August, 1, 10, 17, 23 September, and 2 October (by which time all cannibals had metamorphosed). Each cannibal's age and size (SVL) at metamorphosis were also recorded. For each cannibal, the response variable was the percentage of prey that were siblings. We predicted that if cannibals did not discriminate kin (the null hypothesis), then 25% of prey would be siblings. Proportional data were arcsine–square-root transformed to meet parametric assumptions of normality. An initial analysis of sibship effects indicated no significant differences among sibships in discriminatory ability ($F_{2,17} = 1.06$, $p = .371$). We therefore treated different sibships as replicates and used a one-sample t test to compare the percentage of prey that were siblings with 25%. We used a one-tailed test because a previously published report (Pfennig et al., 1994) indicated that tiger salamander larvae from the same population avoid eating their kin in the laboratory.

Results

Any differences in mortality between kin and non-kin in treatment enclosures could be ascribed to prey preferences of cannibals, and not to variation among families in other sources of mortality because all typical morph larvae survived in control enclosures. As shown in Table 2, the mean percentage of prey that were siblings in treatment enclosures was 20%, which was significantly less than 25%, the value expected if cannibals had eaten siblings and nonsiblings at random ($t = -2.09$, df = 17, $p = .026$; one-tailed, one sample t test on arcsine–square-root transformed data). Thus, kin recognition is expressed in the wild, demonstrating that avoidance of kin cannibalism is not a by-product of laboratory conditions.

Table 2
Evidence that *A. tigrinum* larvae can discriminate kin in the wild

Enclosure number	Cannibal's sibship	Number of larvae eaten	Percentage of prey that were siblings
1	LC	15	20
2	SO	10	40[a]
3	WP	5	20
4	LC	4	0
5	LC	12	17
6	LC	10	10
7	SO	15	20
8	SO	16	13
9	WP	9	33[a]
10	LC	9	33[a]
11	LC	13	15
12	LC	6	0
13	WP	13	23
14	WP	5	20
15	LC	15	20
16	LC	10	10
17	SO	16	25[a]
18	LC	13	39[a]
Mean			20[b]

[a] Cannibals that failed to discriminate kin.

[b] Mean percentage of prey that were siblings was significantly <25%, the value expected if cannibalism were random with respect to kinship.

Experiment 2: Is kin recognition an artifact of sibship-specific variation in escape response?

Methods

We first determined if families varied in escape responses by subjecting similarly sized typical morph larvae from different families to a simulated attack by a cannibal (see Pfennig et al., 1998). We used 95 larvae from 5 Arizona sibships that were housed individually in 22-l tanks filled with 16 l of dechlorinated water. An observer, who was unaware of a larva's sibship, touched the distal tail of each larva with a plastic rod (460 mm long × 10 mm diameter) and assigned each animal to an ordinal score depending on its response to being tapped. If the salamander showed no response, we gave it a score of 0, if the salamander crawled away, we gave it a score of 1, and if the salamander swam away, we gave it a score of 2. We determined if families differed in these responses by using a Kruskal-Wallis nonparametric one-way ANOVA test. We then ranked the families according to their mean escape response scores, from lowest (= slowest) to highest (= fastest).

Next, we determined if family-specific variation in escape response predicts whether a cannibal would ingest kin or non-kin. We evaluated kin discrimination abilities of 63 cannibals from the same 5 families that were used to calculate escape response scores. Cannibals were given a choice of eating a sibling larva or a similarly sized nonsibling larva, the latter being from one of the other four families. We predicted that if kin recognition were an artifact of sibship-specific variation in escape response, then the relative escape response scores of the two families involved should reliably predict whether the cannibal would eat kin or non-kin. For instance, if the cannibal's family were slower than the non-kin's family in escape response, then the escape-response hypothesis would predict that the cannibal should eat (slower) kin instead of (faster) non-kin. We asked how many times this prediction was met for the 20 combinations of cannibal–non-kin families. We tested each cannibal when it was satiated (i.e., when cannibals had been fed another salamander 1–2 days before the test took place) and again when it was hungry (i.e., when cannibals had not been fed another salamander 7–8 days before the test took place). We controlled for hunger because of a previous report (Pfennig et al., 1993) that cannibalistic spadefoot toad tadpoles are less likely to avoid eating siblings when they are hungry than when they have just eaten.

To start an experiment, we put 16 l of dechlorinated tap water into a 22-l aquarium and introduced one cannibal morph and two "stimulus" animals (both typical morph larvae) matched for size. One stimulus animal was the cannibal's sibling, and the other was a nonsibling. Stimulus larvae were

about half the cannibal's SVL, and stimulus animals had been reared apart from cannibals since they were ≤ 2 weeks old; different sibships were completely unfamiliar with each other.

To keep track of kinship identities, we cut a small hole (2–3 mm) in either the dorsal or ventral half of each stimulus animal's fin. To control for effects of these marks, in half the aquaria the test cannibal's sibling was marked dorsally and the nonsibling ventrally, and in the other half the test cannibal's sibling was marked ventrally and the nonsibling dorsally. These marks did not affect larval mortality. An observer who was unaware of the sibship identities of the stimulus animals checked each aquarium at least once every hour between 0800 h and 2000 h and noted when cannibalism had occurred (when a tank mate was consumed) and the tail mark of the surviving stimulus animal. Throughout the experiment, larvae were fed the standard ration of live Artemia daily. Test and stimulus animals were used only once. The response variable was the percentage of prey each cannibal ingested that were siblings. These proportions were arcsine–square-root transformed to meet parametric assumptions of normality.

Results

Although larvae from different families differed significantly in escape responses ($H = 13.72$, df = 4, $p = .008$; Kruskal-Wallis test), relative response scores did not predict reliably whether a satiated cannibal would ingest kin or non-kin. In 20 trials, the slower prey was eaten 11 times and the faster prey was eaten 9 times ($\chi^2 = 0.2$, df = 1, $p = .66$). However, when the cannibal was hungry, escape responses correctly predicted whether kin or non-kin were eaten: in all 20 trials using hungry cannibals, the cannibal ate the slower prey. Thus, the escape-response hypothesis applies in the special case where cannibals are hungry and, presumably, not discriminating kin. However, because the escape-response hypothesis cannot explain avoidance of kin cannibalism when cannibals are satiated, we reject this hypothesis as a general explanation for kin recognition.

Experiment 3: Is kin recognition an epiphenomenon of species recognition?

Methods

The species-recognition hypothesis predicts that cannibals from populations that co-occur with other species of salamanders should have more refined kin discriminatory abilities than cannibals from populations that do not co-occur with other species of salamanders. We tested this prediction by contrasting kin discriminatory abilities of 63 cannibals from 5 Arizona sibships with that for 50 cannibals from 10 Indiana sibships. Cannibals from Indiana occur sympatrically with other species of salamanders, including a congener, *A. texanum*. In contrast, *A. tigrinum* is the sole salamander species found in Arizona.

We tested the discrimination abilities of larvae 7–9 weeks after they had hatched using the testing procedures outlined above in experiment 2. However, we controlled for inter-regional variation in kin discriminatory ability due to differing propensities to produce cannibals. This control was important because, for Arizona larvae, the greater the probability that a larva from a given sibship would develop into a cannibal morph, the more likely the members of that sibship are to discriminate kin (Pfennig et al., 1994). Thus, it might be contended that any between-region variation in kin discriminatory ability may be due to differing propensities to produce cannibals and not to differing exposures to heterospecifics. We therefore contrasted kin discriminatory abilities for sibships across the two regions that had similar propensities to produce cannibals. The propensity of each sibship to produce cannibal morphs was determined by calculating the proportion of 10–20 separate aquaria containing each sibship that produced a cannibal morph (e.g., see Pfennig and Collins, 1993).

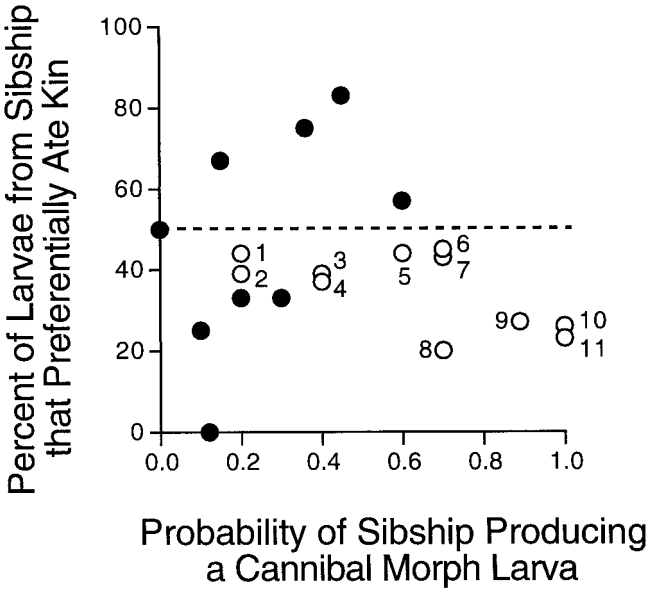

Figure 1
Relationship between the proportion of larvae from 21 different sibships that ate kin and the proportion of cannibals produced by those sibships. Data are from two regions: 11 sibships (open circles) are from 5 different ponds in Arizona (allopatric with other salamander species; disease present) and 10 sibships (dark circles) are from a single pond in Indiana (sympatric with other salamander species; disease absent). Data for 6 of the 11 Arizona sibships (sibships 1–4, 8, 9) are from Pfennig et al. (1994).

Results

Of 50 cannibals tested from the population that occurs sympatrically with other species of salamanders (i.e., Indiana population), 26 (52%) ate kin, but of 63 cannibals tested from the allopatric population (i.e., Arizona population), only 21 (33%) ate kin. However, these inter-regional differences in kin discrimination disappeared once we controlled for inter-regional variation in propensity to produce cannibal morphs: when we restricted our comparison to those Arizona sibships that did not differ from Indiana sibships in propensity to produce cannibal morphs (i.e., sibships 1–5 in Figure 1), cannibals from the two regions did not differ in mean discrimination abilities ($\chi^2 = 2.49$, df = 1, $p = .11$). Therefore, kin recognition was not more pronounced in populations where tiger salamanders are sympatric with other species of salamanders, implying that kin recognition is not an epiphenomenon of species recognition.

Experiment 4: Does kin recognition function in disease avoidance?

Methods

The disease-avoidance hypothesis may be especially applicable to tiger salamanders because this species is often afflicted with deadly disease epidemics (Worthylake and Hovingh, 1989; Pfennig et al., 1991a; Jancovich et al., 1997; Pfennig et al., 1998). Moreover, cannibalism is a mode of disease transmission in this system (Pfennig et al., 1991a; Pfennig et al., 1998). Although the precise causes of these epidemics are not known, disease may be caused by two species of bacteria (*Aci-*

netobacter sp.: Worthylake and Hovingh, 1989; *Clostridium* sp.: Pfennig et al., 1991a) and a virus (*Ambystoma tigrinum* virus: Jancovich et al., 1997).

To determine if kin recognition functions in disease avoidance, we first examined whether families differ in susceptibility to disease. We randomly selected 16 equal-sized, 7-week-old typical morph larvae from each of 6 Arizona sibships that had been reared under similar conditions since birth and placed them individually into a container filled with 3.6 l of dechlorinated tap water. We then exposed eight larvae from each sibship (treatment larvae) to 0.4 l of water from a diseased pond, and eight larvae (control larvae) to 0.4 l of autoclaved water from the same pond. We fed each treatment animal 2 g of liver from a diseased animal (livers of diseased animals have numerous lesions containing pathogenic bacteria; Pfennig et al., 1991a). Control larvae were each fed 2 g of liver from a healthy animal. These control larvae were used to determine if families differed intrinsically in their survival, whereas treatment larvae were used to determine if families differed in susceptibility to disease. Because the pathogens are often highly virulent, the response variable was number of days after the start of the experiment when treatment animals died. We used a one-way ANOVA to determine if individuals from different families varied significantly in time of death.

We then asked if cannibals from diseased and nondiseased populations differ in kin discrimination abilities. The disease avoidance hypothesis predicts that cannibals from diseased populations should avoid eating kin, whereas those from nondiseased populations should eat kin and non-kin indiscriminately. We tested this prediction by contrasting the kin discrimination abilities of cannibals from Arizona and Indiana. Disease epidemics are common in many parts of Arizona, including the White Mountains where our experimental subjects were collected (Collins JP, personal observation). In contrast, disease epidemics have not been reported in Indiana. We conducted this experiment simultaneously with experiment 3 by using 63 cannibals from 5 Arizona sibships and 50 cannibals from 10 Indiana sibships. The response variable was the percentages of prey each cannibal ingested that were siblings.

Next, we tested the critical prediction of the disease avoidance hypothesis: that kin are more infectious than non-kin. We compared disease transmission among kin and non-kin in 1993 using 32 similarly sized cannibals from 4 Arizona sibships and again in 1994 using 30 similarly sized cannibals from 3 additional Arizona sibships. Cannibals were housed individually in aquaria and reared under identical conditions (22°–25°C water temperature and 14 h:10 h photoperiod; they were fed live brine shrimp daily ad libitum). We randomly assigned cannibals to two different prey treatment groups: each cannibal was either fed a single diseased sibling (1993: $n = 17$; 1994: $n = 16$) or a single diseased nonrelative (1993: $n = 15$; 1994: $n = 14$). Prey were similarly sized typical morph larvae that were about half the size of the cannibals. We created diseased prey by housing typical morph larvae inside aquaria with diseased field-caught larvae for 2 h (larvae exposed longer invariably died of disease). In 1993 larvae were exposed to diseased field-caught larvae with symptoms of *Ambystoma tigrinum* virus (Jancovich et al., 1997), whereas in 1994 larvae were exposed to diseased field-caught larvae with symptoms of *Clostridium* bacterial infection (Pfennig et al., 1991a). We anticipated that many cannibals would die because ingestion of a single diseased larva is sufficient to cause mortality due to disease (Pfennig et al., 1991a). Therefore, the response variable was number of cannibals that survived to metamorphosis.

Finally, we tested the prediction that cannibals should prefer nondiseased over diseased prey. We placed 20 satiated cannibals from Arizona individually in 22-l aquaria filled with 16 l of dechlorinated water. Into each tank, we placed two smaller, non-kin, typical morph larvae, one of which was healthy and the other of which was diseased (diseased animals display hemorrhagic septicemia, and they tend to float lethargically on the water surface). An observer recorded which animal (diseased or healthy) was consumed first. We used a chi-square test to compare the number of each type of prey consumed to the number expected (10) if cannibalism were random with respect to prey health.

Table 3
Longevity of cannibals from different sibships when exposed to naturally occurring pathogens

Sibship	Mean (SD) longevity (days)
LC-21	4.2 (0.4)
So2	4.5 (0.5)
LC-16	5.4 (0.5)
OA	5.8 (1.4)
WP7	5.9 (1.3)
LC-23	6.4 (1.2)

Results

In the test to determine whether families differed in susceptibility to disease, all treatment larvae died. However, larvae from different families differed significantly in time of death ($F_{5,44} = 6.23$, $p = .0002$; Table 3). There were even highly significant sibship effects on the time of death among the three LC sibships that were cousins ($F_{2,22} = 16.32$, $p = .0001$; Table 3). That these family-specific differences were likely due to disease and not to intrinsic differences in mortality was suggested by the finding that all control larvae survived. Thus, families differed in susceptibility to disease, and this variation occurred among larvae from the same pond.

Given that the above critical assumption of the disease avoidance hypothesis was true, we next tested this hypothesis in three ways. First, we used a comparative approach to determine if cannibals from diseased and nondiseased populations differed in kin discrimination abilities. We found that once we had controlled for inter-regional variation in propensity to produce cannibal morphs, cannibals from diseased and nondiseased regions did not differ in discrimination abilities (Figure 1, see also experiment 3 results). Therefore, contrary to the prediction of the disease avoidance hypothesis, kin recognition was not more pronounced in diseased populations than in nondiseased populations.

Second, we asked if kin are more infectious to cannibals than are non-kin. We found that in 1993, 5 of 15 (33%) cannibals that ate diseased non-kin died, whereas none of the 17 cannibals that ate diseased kin died ($\chi^2 = 6.716$, df = 1, $p = .009$). In 1994, the results were similar, although not statistically significant: 11 of 14 (79%) cannibals that ate diseased non-kin died, whereas 9 of 16 (56%) cannibals that ate diseased kin died ($\chi^2 = 1.674$, df = 1, $p = .20$). When both years were combined, cannibals of non-kin died at a significantly higher rate than did cannibals of kin (Table 4). Therefore, contrary to the prediction of the disease avoidance hypothesis, diseased non-kin were more infectious than were diseased kin.

Finally, we predicted that if the disease avoidance hypothesis were correct, cannibals should avoid diseased prey. However, when 20 satiated cannibals from the diseased population were housed individually with two smaller larvae, 1 diseased and 1 healthy, 13 cannibals (65%) ate the diseased prey (presumably because they were easier to catch), and 7 cannibals (35%) ate the healthy prey ($\chi^2 = 1.98$, df = 1, $p = .16$). Thus,

Table 4
Fates of cannibals that were fed diseased siblings and diseased non-kin

	Number (%) of cannibals that survived	Number (%) of cannibals that died
Sibling eaters	24 (73)	9 (27)[a]
Non-kin eaters	13 (45)	16 (55)[a]

[a] $\chi^2 = 4.993$, df = 1, $p = .026$.

contrary to the prediction of the disease avoidance hypothesis, cannibals did not avoid diseased prey.

Experiment 5: Is kin recognition kin selected?

Methods

To answer the question of whether kin recognition is kin selected, we asked if avoidance of kin cannibalism satisfies Hamilton's rule (Hamilton, 1964). Hamilton's rule predicts that avoidance of sibling cannibalism will be favored by natural selection if $c/b < ½$, where ½ is the coefficient of relatedness between full siblings. To estimate cost (c) and benefit (b) of kin discrimination, we used various measures of direct and indirect fitness to compare cannibals in experiment 1 that ate <25% kin (discriminators, $n = 13$) with those cannibals that ate ≥25% kin (nondiscriminators, $n = 5$), where 25% kin consumption was the value expected if cannibalism were random with respect to kinship (see Table 2). Discriminators and nondiscriminators were from the same sibships, they were of the same age and initial sizes (mean±SD SVL of discriminators = 38.3±3.9 mm; mean±SD SVL of nondiscriminators = 38.7±1.5; $p = .66$; two-tailed Mann-Whitney test), and they had similar growth rates (Figure 2). We predicted that if discriminators fared significantly better than nondiscriminators in terms of benefit, but if the two types of cannibals did not suffer different costs, then Hamilton's rule would be true (i.e., $c/b < ½$), implying that kin recognition is kin selected.

To estimate benefit of kin discrimination, we compared the number of siblings that survived to metamorphosis for discriminators and nondiscriminators. Note that this benefit is not inevitably positive; for example, if all noncannibalized individuals were to die from starvation or disease before metamorphosis, then this benefit would be zero, and if noncannibalized siblings were more likely than nonsiblings to compete for the same foods, then this benefit could even be negative.

To estimate cost of discrimination, we compared survival, growth rate, and age at metamorphosis for discriminators and nondiscriminators. Our rationale for using these measures was that a cannibal that recognizes and avoids preying on kin thereby provides benefits to its relatives, but the cannibal may suffer the personal cost of diminished growth or survival by forgoing a meal (Crump, 1992). Growth rate and age at metamorphosis are both likely to be sensitive to reduced food intake [such as what may be experienced by a discriminating cannibal (e.g., see Lannoo et al., 1989)], and both correlate significantly with several components of fitness in amphibians, such as adult survival (Pfennig et al., 1991b) and age at first reproduction (Semlitsch et al., 1988).

Results

As indicated in Figure 2, the benefit of kin discrimination (b) was large: discriminators had more than twice as many siblings survive to metamorphosis than did nondiscriminators ($p = .004$; two-tailed t test). In contrast, the cost of kin discrimination was small (Figure 2). Discriminators and nondiscriminators had equal survival (all survived to metamorphosis), growth rate (SVL; discriminators: 8.87±3.92 mm, nondiscriminators: 9.75±5.05 mm; $p = .78$; two-tailed Mann-Whitney test), and age at metamorphosis (discriminators: 137±8 days; nondiscriminators: 132±12 days; $p = .45$; two-tailed Mann-Whitney test). Therefore, the small costs that cannibals incurred by discriminating kin were likely outweighed by the important benefits that relatives received by not being eaten. Thus, Hamilton's rule is likely to be true in our system (i.e., $c/b < ½$), suggesting that kin recognition is maintained by kin selection.

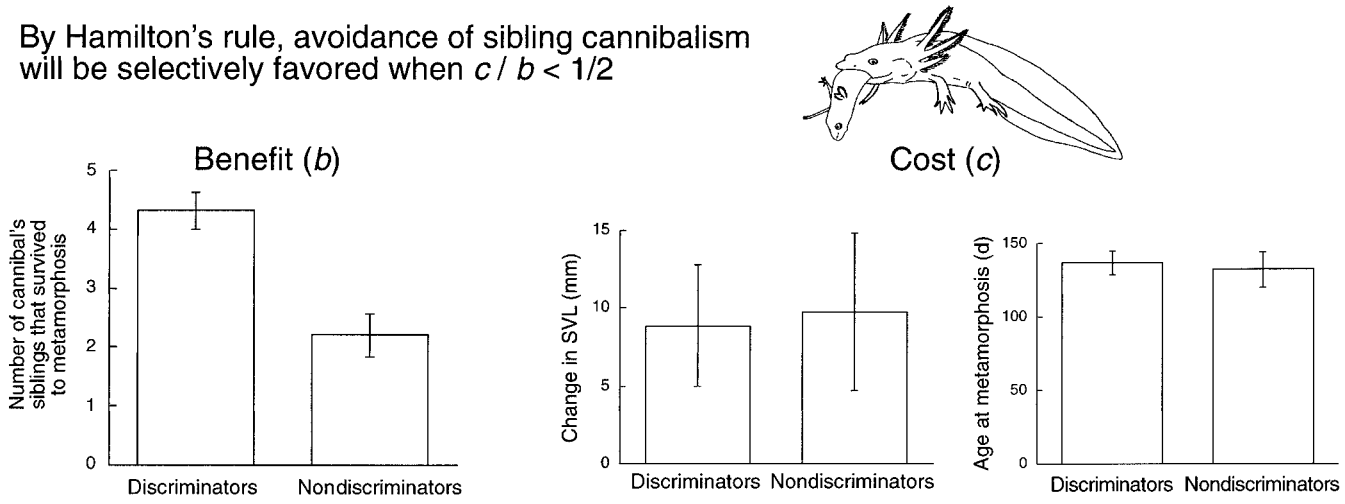

Figure 2
A test of Hamilton's rule for avoidance of sibling cannibalism in tiger salamanders. Benefit of kin discrimination (left graph) was estimated by comparing number of siblings that survived to metamorphosis for cannibals that ate <25% kin (discriminators, $n = 13$) and for cannibals that ate ≥ 25% kin (nondiscriminators, $n = 5$), where 25% kin consumption was the value expected if cannibalism were random with respect to kinship. Costs of kin discrimination were estimated by comparing growth rate (center graph) and age at metamorphosis (right graph) for the same two types of cannibals. Discriminators produced significantly more siblings than did nondiscriminators, but discriminators and nondiscriminators did not differ in growth rate or age at metamorphosis.

DISCUSSION

Our study helps clarify the function of kin recognition in cannibalistic species. We found no evidence that kin recognition is epiphenomenal in tiger salamanders (experiments 1–3). In particular, we found that (1) kin recognition was expressed in a natural setting, demonstrating that avoidance of kin cannibalism is not an artifact of laboratory conditions, (2) escape responses did not reliably predict whether a satiated cannibal would ingest kin or non-kin, implying that kin recognition is not a by-product of sibship-specific variation in escape responses, and (3) kin recognition was not most pronounced in populations where tiger salamanders co-occur with other species of salamanders, ruling out species recognition. Moreover, it is unlikely that cannibals simply avoid eating salamanders they are reared with, as opposed to kin per se. Previous experiments show that cannibals are as effective at discriminating between first cousins and nonrelatives as they are discriminating between siblings and nonrelatives, despite never having been exposed to cousins (Pfennig et al., 1994). Thus, although Grafen (1990) has claimed that many examples of kin discrimination are artifacts of some other recognition system, such as species or group-member identification, kin recognition is not epiphenomenal in cannibalistic tiger salamanders. Instead, kin recognition appears to be maintained by natural selection because it enhances the cannibal's inclusive fitness.

Given that kin recognition is maintained by natural selection, we asked whether it enhances the direct or indirect component of a cannibal's inclusive fitness. A possible direct benefit is disease avoidance (Pfennig et al., 1993). This hypothesis assumes that kin are more infectious than non-kin. However, we found that unrelated prey were more likely than related prey to transmit pathogens to cannibals (experiment 4). This finding, which was based on 2 years of data using two different types of pathogens, was surprising in light of recent evidence that genetically similar individuals are more likely to infect each other with pathogens than are genetically dissimilar individuals (Black, 1994; Pfennig et al., 1998; Shykoff and Schmid-Hempel, 1991). It is unclear why non-kin would be more likely to transmit pathogens to each other. Perhaps an individual's kin are less likely to carry diseases to which the individual is susceptible but for which the individual would not have previously developed immune responses. Regardless of why non-kin are more infectious than kin, this finding weakens the disease avoidance hypothesis.

Two other results from experiment 4 imply that kin recognition does not function in disease avoidance. First, when we controlled for different propensities to produce cannibal morphs, we found that cannibals from diseased populations were no more discriminating of kin than were cannibals from a nondiseased population (Figure 1). Second, when offered a choice of diseased prey and healthy prey, cannibals from diseased populations showed no preference, suggesting an absence of strong selection to avoid disease.

If kin recognition is neither an epiphenomenon nor a means for cannibals to obtain direct inclusive fitness benefits, then kin selection furnishes a compelling explanation for the evolutionary maintenance of kin recognition. By Hamilton's rule (Hamilton, 1964), avoidance of sibling cannibalism will be favored when the ratio of the cost incurred by the cannibal to the benefit accrued by the recipient (i.e., c/b) is $< \frac{1}{2}$. We were able to assess the relative values of c and b using data from an experimental population in a natural setting. Such estimates of lifetime fitness often suffice to test Hamilton's rule (e.g., Emlen and Wrege, 1989; Grafen, 1984; Reyer, 1984).

We inferred that Hamilton's rule is satisfied for avoidance of sibling cannibalism (experiment 5). In particular, we found that discriminating cannibals received substantial indirect fitness benefits by not eating kin, but there was no evidence that these cannibals subsequently paid a cost by avoiding kin cannibalism (Figure 2). Therefore, Hamilton's rule is likely to be satisfied in our system, implying that kin recognition in cannibalistic tiger salamanders is favored by kin selection. Thus, our study reinforces the view (Blaustein and O'Hara, 1982; Pfennig, 1999; Pfennig and Collins, 1993; Pfennig et al., 1993, 1994; Sherman, 1981; Wade, 1980; Waldman, 1991; Walls and Blaustein, 1995; Walls and Roudenbush, 1991) that kin selection is important for maintaining kin recognition in cannibalistic individuals.

It might be contended that our estimate of the benefit of discrimination was inflated, since only one cannibal was in each of our field cages. In particular, if other unrelated cannibals were present, they might have eaten the focal cannibal's noncannibalized kin, thereby depressing the number of kin that ultimately survived to metamorphosis. This implies that in ponds where cannibals are present in high frequencies, Hamilton's rule may not be satisfied. Thus, the benefit of kin discrimination may differ in different environments.

It is also possible that the cost of kin discrimination varies in different environments. For instance, a discriminating cannibal may pay a substantial cost for discriminating kin in populations where disease epidemics occur because preferential cannibalism of non-kin appears to increase a cannibal's risk of acquiring deleterious pathogens (see experiment 4 results). Despite this cost, however, cannibals from diseased populations were no less discriminating than those from nondiseased populations (e.g., compare in Figure 1 the level of kin discrimination among cannibals from Arizona, where diseases are prevalent, with that among cannibals from Indiana, where diseases are apparently absent). Thus, the indirect inclusive fitness benefits of kin recognition must be substantial for this behavior to be maintained by natural selection even where diseases are prevalent.

In conclusion, despite the widespread view that kin selection is the primary agent maintaining kin recognition, relatively few studies have examined the fitness consequences of kin recognition. Although our results implicate kin selection as the overriding reason that cannibalistic tiger salamanders discriminate kin, it is important to consider whether kin recognition is an artifact or epiphenomenon of some other factor that operates instead of or in addition to kin selection. Only by considering alternative hypotheses are we likely to succeed in determining why organisms recognize their kin.

We thank Karin Pfennig, Kern Reeve, Paul Sherman, Ron Ydenberg, and two anonymous referees for helpful comments on the manuscript, Rick Howard and Howard Whiteman for collecting Indiana salamanders, Eric Hoffman, Larry Nienauber, Karin Pfennig, Jim Roth, and Kristine Ziemba for laboratory and field assistance, and the White Mountain Apache Indian tribe and the Arizona Game and Fish Department for collecting permits. This research was supported by grants from the U. S. National Science Foundation to D. P. and J. C.

REFERENCES

Barnard C, 1991. Kinship and social behaviour: the trouble with relatives. Trends Ecol Evol 6:310–312.

Black FL, 1994. An explanation of high death rates among New World peoples when in contact with Old World diseases. Perspect Biol Med 37:292–307.

Blaustein AR, Bekoff M, Byers JA, Daniels TJ, 1991. Kin recognition in vertebrates: what do we really know about adaptive value? Anim Behav 41:1079–1083.

Blaustein AR, Bekoff M, Daniels TJ, 1987. Kin recognition in vertebrates (excluding primates): mechanisms, functions, and future research. In: Kin recognition in animals (Fletcher DJC, Michener CD, eds). New York: John Wiley; 333–357.

Blaustein AR, O'Hara RK, 1982. Kin recognition in *Rana cascadae* tadpoles: maternal and paternal effects. Anim Behav 30:1151–1157.
Brown JL, 1987. Helping and communal breeding in birds: ecology and evolution. Princeton, New Jersey: Princeton University Press.
Carlin NF, 1989. Discrimination between and within colonies of social insects: two null hypotheses. Neth J Zool 39:86–100.
Collins JP, Cheek JE, 1983. Effect of food and density on development of typical and cannibalistic salamander larvae in *Ambystoma tigrinum nebulosum*. Am Zool 23:77–84.
Collins JP, Zerba KE, Sredl MJ, 1993. Shaping intraspecific variation: development, ecology and the evolution of morphology and life history variation in tiger salamanders. Genetica 89:167–183.
Crump ML, 1992. Cannibalism in amphibians. In: Cannibalism. Ecology and evolution among diverse taxa (Elgar MA, Crespi BJ, eds). Oxford: Oxford University Press; 256–276.
Emlen ST, Wrege PH, 1989. A test of alternative hypotheses for helping behavior in white-fronted bee-eaters of Kenya. Behav Ecol Sociobiol 25:303–319.
Fletcher DJC, Michener CD (eds), 1987. Kin recognition in animals. New York: John Wiley.
Freeland WJ, 1983. Parasites and the coexistence of animal host species. Am Nat 121:223–236.
Gamboa GJ, Reeve HK, Holmes WG, 1991. Conceptual issues and methodology in kin-recognition research: a critical discussion. Ethology 88:109–127.
Grafen A, 1984. Natural selection, kin selection, and group selection. In: Behavioural ecology: an evolutionary approach, 2nd ed. (Krebs JR, Davies NB, eds). Sunderland, Massachusetts: Sinauer; 62–84.
Grafen A, 1990. Do animals really recognize kin? Anim Behav 39:42–54.
Hamilton WD, 1964. The genetical evolution of social behaviour. I, II. J Theor Biol 7:1–52.
Hepper PG, ed, 1991. Kin recognition. Cambridge: Cambridge University Press.
Hoffman EA, Pfennig DW, 1999. Proximate causes of cannibalistic polyphenism in larval tiger salamanders. Ecology 80:1076–1080.
Jancovich JK, Davidson EW, Morado JF, Jacobs BL, Collins JP, 1997. Isolation of a lethal virus from the endangered tiger salamander *Ambystoma tigrinum stebbinsi*. Dis Aquat Org 31:161–167.
Lannoo MJ, Lowcock L, Bogart JP, 1989. Sibling cannibalism in noncannibal morph *Ambystoma tigrinum* larvae and its correlation with high growth rates and early metamorphosis. Can J Zool 67:1911–1914.
Loeb MLG, Collins JP, Maret TJ, 1994. The role of prey in controlling expression of a trophic polymorphism in *Ambystoma tigrinum nebulosum*. Funct Ecol 8:151–158.
Møller AP, Dufva R, Allander K, 1993. Parasites and the evolution of host social behavior. Adv Study Behav 22:65–102.
Pedersen SC, 1991. Dental morphology of the cannibal morph in the tiger salamander, *Ambystoma tigrinum*. Amphib-Reptilia 12:1–14.
Pfennig DW, 1990. 'Kin recognition' among spadefoot toad tadpoles: a side-effect of habitat selection? Evolution 44:785–798.
Pfennig DW, 1997. Kinship and cannibalism. BioScience 47:667–675.
Pfennig DW, 1999. Cannibalistic tadpoles that pose the greatest threat to kin are most likely to discriminate kin. Proc R Soc Lond B 266: 57–61.
Pfennig DW, Collins JP, 1993. Kinship affects morphogenesis in cannibalistic salamanders. Nature 362:836–838.
Pfennig DW, Ho SG, Hoffman EA, 1998. Pathogen transmission as a selective force against cannibalism. Anim Behav 55:1255–1261.
Pfennig DW, Loeb MLG, Collins JP, 1991a. Pathogens as a factor limiting the spread of cannibalism in tiger salamanders. Oecologia 88: 161–166.
Pfennig DW, Mabry A, Orange D, 1991b. Environmental causes of correlations between age and size at metamorphosis in *Scaphiopus multiplicatus*. Ecology 72:2240–2248.
Pfennig DW, Reeve HK, Sherman PW, 1993. Kin recognition and cannibalism in spadefoot toad tadpoles. Anim Behav 46:87–94.
Pfennig DW, Sherman PW, 1995. Kin recognition. Sci Am 272:98–103.
Pfennig DW, Sherman PW, Collins JP, 1994. Kin recognition and cannibalism in polyphenic salamanders. Behav Ecol 5:225–232.
Polis GA, 1981. The evolution and dynamics of intraspecific predation. Annu Rev Ecol Syst 12:225–251.
Powers JH, 1907. Morphological variation and its causes in Ambystoma tigrinum. Stud Univ Nebraska 7:197–274.
Reyer H-U, 1984. Investment and relatedness: a cost/benefit analysis of breeding and helping in the pied kingfisher. Anim Behav 32: 1163–1178.
Sadler LM, Elgar MA, 1994. Cannibalism among amphibian larvae: a case of good taste. Trends Ecol Evol 9:5–6.
Semlitsch RD, Scott DE, Pechmann JHK, 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology 69:184–192.
Sherman PW, 1981. Reproductive competition and infanticide in Belding's ground squirrels and other animals. In: Natural selection and social behavior (Alexander RD, Tinkle DW, eds). New York: Chiron Press; 311–331.
Sherman PW, Reeve HK, Pfennig DW, 1997. Recognition systems. In: Behavioural ecology: an evolutionary approach, 4th ed. (Krebs JR, Davies NB, eds). Oxford: Blackwell Scientific; 69–96.
Shykoff JA, Schmid-Hempel PS, 1991. Parasites and the advantage of genetic variability within social insect colonies. Proc Roy Soc Lond B 243:55–58.
Stuart RJ, 1991. Kin recognition as a functional concept. Anim Behav 41:1093–1094.
Wade MJ, 1980. An experimental study of kin selection. Evolution 34: 844–855.
Waldman B, 1991. Kin recognition in amphibians. In: Kin recognition (Hepper P, ed). Cambridge: Cambridge University Press; 162–219.
Walls SC, Blaustein AR, 1995. Larval marbled salamanders, *Ambystoma opacum,* eat their kin. Anim Behav 50:537–545.
Walls SC, Roudenbush RE, 1991. Reduced aggression toward siblings as evidence of kin recognition in cannibalistic salamanders. Am Nat 138:1027–1038.
Worthylake KM, Hovingh P, 1989. Mass mortality of salamanders (*Ambystoma tigrinum*) by bacteria (*Acinetobacter*) in an oligotrophic seepage mountain lake. Great Basin Nat 49:364–372.